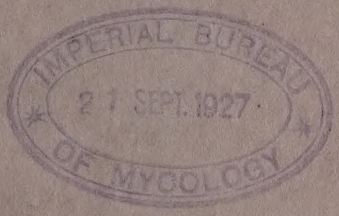
Bulletin No. 311 June, 1927

THE BLACK-ROOT DISEASE OF RADISH



PURDUE UNIVERSITY
AGRICULTURAL EXPERIMENT STATION
LAFAYETTE, INDIANA

Bulletin No. 311 June, 1927

THE BLACK-ROOT DISEASE OF RADISH

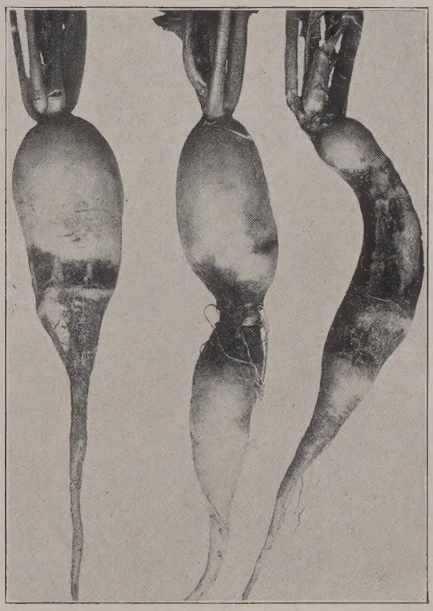


Fig. 1. Radish black-root, a disease which is caused by a soil fungus, Aphanomyces raphani n. sp. Types of infection on the White Icicle variety which is very susceptible to the disease: Plant at the left shows the blackened, fissured surface of the lower portion of the root; plant in the center shows a blackened constricted region as a result of the arrested growth of the infected portion of the root; plant at the right shows a severe case of the disease in which the entire radish is involved.

PURDUE UNIVERSITY
AGRICULTURAL EXPERIMENT STATION
LAFAYETTE, INDIANA

SUMMARY

Radish black-root is characterized by a deeply penetrating, blackened discoloration of the root tissues.

The causal fungus produces a white, prostrate mycelial mat on potato dextrose agar. It grows best at 23° to 27° C. (73°—81° F.).

Zoospores are formed in a single row in profusely branched zoosporangia which discharge their zoospores from one or more spirally curled, tapering, lateral hyphae. Antheridia are one to three to each oogonium. The oospores are thick-walled. Apparently the fungus has not been previously described and is herein designated as *Aphanomyces raphani* n. sp.

The fungus usually enters the host tissues at the natural wound made where the secondary root emerges from the tissues of the primary root. In the globe varieties most of the edible portion is hypocotyl rather than root and bears no secondary roots.

The hyphae are intercellular and do not cause a softening of the invaded host tissues. Microscopically, the location of the fungus in the host tissues is shown by the presence of dark-brown masses in the intercellular spaces and by the brown discoloration of the adjacent cell walls.

The pathogenicity of the causal fungus has been repeatedly proved by greenhouse inoculations and field test plots. Of the 30 seedsmen's varieties of radishes tested, only the White Chinese variety has shown any consistent resistance to the disease.

The disease is more severe on the white, especially the long white varieties, than on the red types. The red globe varieties often escape serious injury.

No host other than radish has been found.

The causal organism overwinters in the soil and may be spread by infested soil. It is not seed-borne.

Early planting to avoid the high temperatures which favor the fungus and the use of the red globe type of radishes where the soil is badly infested are suggested as partial control measures for the disease.

THE BLACK-ROOT DISEASE OF RADISH

JAMES B. KENDRICK[1]

The disease of radish (*Raphanus sativus* L.) known as black-root has been under observation in Indiana since 1920. The seriousness of the disease, its wide distribution, and the existing confusion in the literature concerning the identity of the causal fungus led to this study of the disease.

Observations and histological studies have shown that the causal organism usually enters the radish plant at the natural wound made where the secondary root emerges from the primary root tissues. The globose type of radishes is injured less severely than the icicle type because of the relative absence of secondary roots on the edible portion of the former. Resistance has been shown by the variety White Chinese.

The causal fungus of radish black-root has been determined to be a species of Aphanomyces, and the fungus *Pythium aphanidermatum* (Edson) Fitz., to which the disease has been attributed, has not been found associated with the disease. A comparison of the fungus causing black-root with the descriptions of the other species of Aphanomyces indicates that it is an undescribed species for which the name *Aphanomyces raphani* has been proposed (13).

HISTORY AND OCCURRENCE OF BLACK-ROOT

Apparently the first report of radish black-root was that of Barrett (2) from Illinois in 1912. He briefly characterized the disease and assigned the causal fungus to the species *Aphanomyces laevis* deBary. In 1915, Edson (6, p. 162) reported the occurrence of a black-root disease of radishes in the District of Columbia, Maryland, Virginia, Long Island, Illinois, and Wisconsin. He considered the causal agent of the radish disease with which he worked to be an undescribed fungus, which he later named *Rheosporangium aphanidermatus* (7). However, he states that the disease is not distinguishable in external appearances from that described by Barrett (2) and suggests the possibility that there may be two very similar but distinct diseases of radish. Following the work of Edson (7), the causal fungus of the black-root disease was generally reported in the literature as *Rheosporangium aphanidermatum* Edson. This organism was later placed in the genus Pythium (8) and in recent reports the disease has been generally attributed to *Pythium aphanidermatum* (Edson) Fitz. Link and Gardner (15) found radish black-root to be of considerable importance in the market, especially on the white radishes. The records of the Fed-

[1] The writer wishes to acknowledge the helpful advice and criticism of Dr. Max W. Gardner at whose suggestion this work was undertaken and he is indebted to Prof. H. S. Jackson for suggestions.

eral Plant Disease Survey show that the disease has been reported from Illinois, Indiana, Kansas, Michigan, Mississippi, New York, Pennsylvania, South Carolina and Wisconsin.

Radish black-root was received from several sections of Iowa in 1925 by the writer. The disease has been observed in home as well as market gardens in Indiana every year since 1920. In 1923, the disease was observed in eight home gardens in one section of Lafayette, and specimens of black-root were sent in from market gardens near New Albany and Evansville, Indiana. The disease occurs annually in some of the large market gardens near Indianapolis.

Radish black-root has been observed in home gardens in Lafayette, Indiana, as early as May 16, and has continued to appear throughout the summer where successive plantings of radishes have been made in diseased soil. The disease appears to be more severe on later plantings than on the early plantings of radishes, indicating that a warm temperature favors its development more than the cool temperature of early spring.

BLACKENING OF EDIBLE TISSUE RUINS RADISHES FOR TABLE USE

The wide distribution of the radish black-root disease and the fact, to be brought out later, that the causal fungus persists in the soil for many years makes this disease a serious problem in many home and market gardens. The blackened condition of the crisp edible tissues renders the radishes unsightly and unsuitable for table use. The disease is especially severe on the White Icicle variety, which is perhaps the most widely grown of the long, white varieties by both home gardeners and commercial radish growers. Because of this disease many home as well as market gardeners have ceased to grow the long, white radishes.

Instances have been observed in Lafayette gardens where 100 per cent of the radish crop in a garden was destroyed by this disease. One home garden in Lafayette was under observation for four years, during which time very few white radishes were secured that were suitable for table use. In this same garden, however, an almost normal crop of the red globe type of radishes was produced each year and the long, red type was less severely injured than were the white varieties. Many of the large market growers in the vicinity of Indianapolis have reported the loss of their entire crop of radishes when the long, white varieties were grown. The importance of this disease from the standpoint of the home gardener as well as the small commercial grower cannot be over-estimated. The available space for the growing of this crop is usually limited, and once the soil becomes infested it is hazardous to attempt to grow certain types of this popular table delicacy.

EDIBLE PORTION OF THE RADISH LARGELY HYPOCOTYL RATHER THAN ROOT

The writer is indebted to Dr. E. J. Kraus, of the University of Wisconsin, for information concerning the gross morphology of the radish. The enlarged edible portion of the long type of radish is composed of both hypocotyl and root tissue. The upper part of the enlarged portion which does not bear secondary roots is hypocotyl, and the lower portion is the true root from which two opposite rows of secondary roots emerge. A somewhat similar anatomical structure is found in turnips, mangels (Percival, 17, pp. 355, 362, 372), and sugar beets (Artschwager, 1, p. 144).

In the globe type of radishes the enlarged edible portion is almost entirely hypocotyl tissue while the lower long, slender portion bearing numerous secondary roots is the true root. Externally, the distinction between root and hypocotyl tissue is denoted approximately by the distribution of the secondary roots, since the portion which bears secondary roots is true root, while the absence of secondary roots generally denotes hypocotyl tissue.

However, since the enlarged edible portion of the radish is generally known as the root, it will be so designated in this publication. The true root of the globe type of radish will be referred to as the tap-root.

ROOTS OF ALL AGES SUSCEPTIBLE TO INFECTION

Radish black-root is primarily a disease of the roots, although, in seedlings, other organs are attacked. There has been no evidence that any form of damping-off results from the fungus causing this disease.

The disease in general is characterized by the blackened condition of the invaded tissues. Infection has been observed on roots as small as two millimeters in diameter, and incipient lesions have been noted on roots up until the time they are full grown. This disease should not be confused with small, sunken, black lesions of downy mildew (*Peronospora parasitica*) on red radish roots reported from Indiana in 1921 (9). These are usually located on the upper end of the edible portion of the root, whereas, the black-root lesions are rarely found on this portion.

LESIONS MORE PRONOUNCED ON LONG WHITE THAN ON RED RADISHES

The symptoms of the disease are more pronounced on the long, white type of radishes than on the long red and the globe types. As has been reported in a previous publication (12), primary infection usually takes place at the base of secondary roots (Fig. 2 D, Fig. 4 C, and Fig. 11 B). The

first visible evidence of the disease is a small, irregular, steel-gray to black area in the surface tissues immediately surrounding the base of the secondary root (Fig. 4 C). In the early stages of the disease, only the layers of tissue immediately under the epidermis are involved. Often dark-colored strands or lace-like patches extending out from the infection center are visible under the epidermis. The blackened tissue immediately under the transparent epidermal tissues gives the lesion a steel-gray color. As the lesions become older and larger the epidermal tissues are also invaded and blackened.

Fig. 2. Symptoms of radish black-root.

A. White Icicle radish showing three blackened diseased areas with a cracked or fissured surface.
B. White Icicle radish showing a blackened constriction as a result of arrested growth in the diseased area.
C. A young White Icicle radish with a large lesion at a clump of secondary roots and several incipient lesions at the bases of other secondary roots. Upper or hypocotyl portion free from lesions. Enlarged x 2.
D. Incipient lesions at the bases of secondary roots on a young radish of the long red type. Upper or hypocotyl portion free from lesions.
E. Globe type of red radish showing incipient lesions on the long slender root at secondary root bases. Swollen hypocotyl portion free from lesions.

Often on young roots incipient lesions are numerous (Fig. 2 D), and these enlarge and coalesce as the root develops, giv-

ing the entire root a black appearance. Incipient lesions are rarely if ever found on the upper enlarged portion of the radish. A diseased condition of this portion usually results from the penetration of the fungus up through the tissues from lesions of early inception in the root below.

A single lesion near the center of a long or oblong root in the early stages of its development often causes a dark-colored, greatly-constricted area on the mature radish (Fig. 1 B, Fig. 2 B, and Fig. 4 A). When infection takes place in the later stages of root enlargement, the spots appear as dark irregular blotches on the surface, or the root is girdled by irregular bands with only slight evidence of constriction. It is not uncommon to find the surface of the lesions longitudinally cracked or fissured, due to the failure of the diseased tissue to keep pace with the growth of the root (Fig. 1 A and C, Fig. 2 A, Fig. 3 A, and Fig. 4 B).

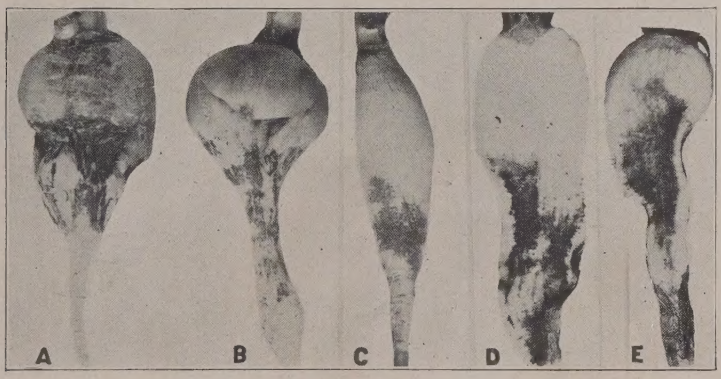


Fig. 3. Distortions and internal blackening of radishes caused by the black-root disease.

A. Oval red radish showing the type of lesion which usually results from infection on one side at the base of the enlarged part of the root. The growth of the infected side has been arrested and the surface tissues are blackened and cracked.

B. Oval red radish showing the arrested growth of the entire lower part of the root due to early general infection. Such lesions are superficial as a rule and the internal tissues are not blackened.

C. White Icicle radish showing the diffused metallic black blotches characteristic of the early stages of the disease.

D. Longitudinal section of a severely infected White Icicle radish root showing depth of penetration of the lesion and the lighter colored healthy tissues interspersed with the black diseased tissues.

E. Longitudinal section of a diseased White Icicle radish. The blackening of the inner tissues shows that the disease may penetrate upward through the hypocotyl tissues from lower points of infection on the true root.

The disease is more commonly confined to the lower half of the enlarged root of the long type of radishes. In many cases, the entire lower half of the root is entirely blackened with the surface showing extensive longitudinal cracking (Fig. 3A). This type of infection often causes the lower half of the root

to be constricted and elongated with a rough, cracked surface (Fig. 3 B).

EDIBLE PORTION OF GLOBE VARIETIES OFTEN ESCAPES INFECTION

Incipient lesions on the globe type of radish do not differ from those on the long type. However, since the enlarged edible portion of the globe type of radish is usually devoid of avenues of infection (secondary roots), the disease is often confined to the tap-root (Fig. 2 E). This fact makes the disease less conspicuous than on the long type in which avenues of infection are present on the lower half or two-thirds of the enlarged edible portion of the root.

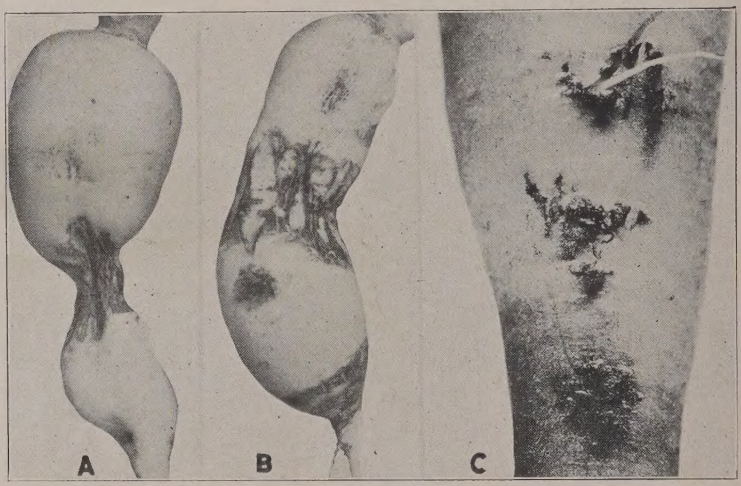


Fig. 4.

A. French Breakfast radish with a black, fissured, lesion of early inception causing constriction as a result of arrested growth of the diseased area. Enlarged x 1½.
B. French Breakfast radish showing lesions near the middle and lower part of the enlarged root. The upper lesion, which is of early inception, shows extensive cracking and has caused a curvature of the root while the lower lesion, which is of later inception, shows only slight cracking and no marked dwarfing of the root. Enlarged x 1½.
C. Incipient lesions at bases of secondary roots on young White Icicle radish root. Enlarged x 5.6.

Early infection about the secondary roots, near the juncture of the enlarged edible portion of the globe type of radish and the small slender tap-root, often produces a blackened condition of the lower end of the enlarged edible portion. In severe cases, the entire edible part of the radish may become involved. Infection often takes place at the juncture of the small root and the enlarged edible part of the radish on one side only. In such cases, the edible portion is flattened and blackened in appearance on the infected side.

The disease is usually more severe on the white globe than on the red globe radishes, due to a more rapid penetration of the fungus into the enlarged edible portion of the former from the tap-root below. Instances have been noted where the white globe radishes were severely damaged by this disease.

INTERIOR TISSUES BLACKENED BUT NOT SOFTENED

In the early stages of the disease, only the tissues immediately under the epidermis are involved, but in the more advanced stages, the causal fungus penetrates all of the internal tissues (Fig. 3 D and E). A cross or longitudinal section of a radish root in an advanced stage of the disease shows irregular blackened areas permeating the tissues in all directions from the point of infection (Fig. 3 D and E). Advancing edges of the lesion, when examined externally or internally, show slender dark strands of infected tissue extending irregularly out through the white sound tissues (Fig. 3 C and D). Even in the more advanced stages of the disease, small irregular areas of healthy tissue are intermingled with the dark colored diseased tissues (Fig. 3 D), but in extreme cases, the tissues are uniformly blackened. The invading parasite does not cause a rot or disintegration of the tissues, but its presence between the cells causes the cell-walls to assume a dark-brown to black color. In the early stages of the disease the invaded tissues remain firm, but the advance of the causal fungus is followed closely by bacteria which may cause a softening of the tissues.

DISEASE MAY ATTACK SEEDLINGS

In greenhouse inoculations and field tests, the disease has been found in the hypocotyl, cotyledon, petiole, and true leaves of seedling radishes. Dark colored streaks extending from the hypocotyl up through the cortex of the petiole into the mid-rib and larger veins of cotyledon and first leaf have been observed (Fig. 5 A). From these regions the causal fungus has been isolated. In some cases, a lateral curvature of the leaf results from mid-rib infection (Fig. 5 A). General infection of large portions of the cotyledon is manifested by scattered dots or small internal dark areas visible through the epidermis (Fig. 5 B). Petiole and leaf infection causes a yellowing and ultimate death of the leaf. Since, in this type of the disease, the blackening has not been traced from the root up through the hypocotyl to the leaves, it is assumed that such infection takes place in the hypocotyl near the base of the petioles.

A diseased condition of the hypocotyl in the seedling stage is not uncommon, especially when the plants are grown in a crowded condition, and, as a result of the infection, the development of the hypocotyl is arrested while the leaf and root

Fig. 5. Internal invasion of hypocotyl, cotyledons, and true leaves in seedlings grown in sterilized inoculated soil.

A. Internal black streaks extending from upper hypocotyl through the outer cortical tissues of the petiole into the mid-rib of the first true leaf, causing a lateral curling of the latter. Enlarged x 2.

B. Cotyledon of seedling radish showing the black streaks extending up the petiole and the small, dark, sunken, infected spots on the upper surface of the lamina. Enlarged x 3½.

development continues to a certain extent. The result is a rather long, slender, constricted dark hypocotyl with stunted leaves and roots. Such plants make a very slow growth and are usually crowded out by the more vigorous plants. This type of the disease has rarely been noted in the field when the plants were properly spaced.

CAUSAL FUNGUS ISOLATED AND GROWN IN PURE CULTURE

Considerable difficulty has been encountered in isolating the causal fungus of radish black-root because of the presence of bacteria in the diseased tissues. Diseased roots were surface sterilized with a 1-1000 solution of mercuric chloride, and fragments of the inner diseased tissue were cut out with a flamed scalpel and planted in poured plates of dextrose and potato dextrose agars. These plantings were usually overrun with bacteria, especially when taken from old lesions. The use of acidified agar and different incubation temperatures proved of no special value in obtaining a growth of the fungus free from bacteria.

In the course of these studies, it has been found, however, that if fragments of diseased tissue were removed aseptically from the advancing edge of lesions of recent origin and planted in poured plates of potato dextrose agar which were then

incubated at 23° to 27° C. for two days, a small percentage of such plantings would usually be free from bacteria and yield a pure culture of the black-root fungus.

A higher percentage of successful isolations was secured from diseased radishes grown in sterilized inoculated soil than from diseased radishes grown in infested field soil. The fungus was readily isolated from diseased petioles and leaves by sterilizing the surface in mercuric chloride and planting small fragments of diseased tissue in agar poured plates (Fig. 6 A). In such isolations, little or no trouble was experienced with bacterial contamination.

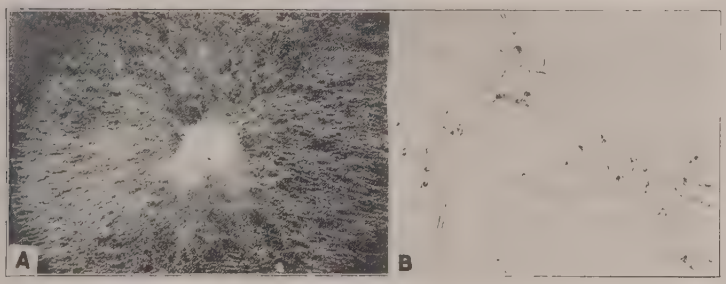


Fig. 6. Characteristic development of the vegetative mycelium of the black-root fungus.

A. A three day old colony on potato dextrose agar. The cobwebby mycelium has developed from a small segment of a diseased petiole placed on the medium. Enlarged x 3.

B. Vegetative hyphae showing characteristic, short, much branched, lateral hyphae, arising at right angles to the main hypha. Water culture. Photomicrograph x 35.

In artificial culture, the growth of the fungus is greatly retarded in the presence of bacteria. Apparently soil bacteria follow very closely the progress of the fungus through the root tissues and the greatest difficulty in isolating the causal fungus of radish black-root is to get it free from these bacteria. When the organism is secured free from bacterial contamination, it grows rapidly in culture.

FUNGUS GROWS WELL ON SOLID AND LIQUID MEDIA

The causal fungus of radish black-root grows well on dextrose and potato dextrose agars, but on corn meal agar, only slight growth occurs. On potato dextrose agar, the mycelium is white, prostrate, and slightly curled (Fig. 6 A). In very young agar cultures, there is a slight tendency towards aerial growth. This aerial growth soon subsides, the mycelium remains prostrate, and the surface of the agar is usually covered in five to eight days with a rather thick, tough mycelial mat. This mat becomes so tough that a strong needle is necessary to cut out small segments for transfers. On slants of corn meal agar the fungus produces a white, thin,

prostrate, web-like growth on the surface of the substratum. The slanted surface is rarely ever completely covered and a tough mat is not formed.

Oospores are formed in the upper layer of the substratum on agar cultures seven to ten days old. Only sparse oospore production has been observed on potato dextrose agar, but in ten-day-old cultures on corn meal agar, an abundance of oospores are produced. Restricted zoosporangium formation has been observed on very moist potato dextrose and corn meal agars.

LIQUID MEDIA FAVOR PRODUCTION OF ZOOSPORES

For morphological studies of the fungus, liquid cultures have been found more suitable. Radish root decoction made by placing small sections of young radish roots in test tubes of distilled water and sterilizing in the autoclave has proved very satisfactory as a medium for typical vegetative growth and sporulation. For use, a test tube of this decoction was poured into a sterile Petri plate, small fragments of mycelium from a young agar culture were added, and the plate was incubated at 25° C. In 3 to 12 hours, such cultures usually showed abundant zoospore production and in 24 to 48 hours moderate mycelial growth had taken place. After three days, oogonia and antheridia appeared and in five to eight days oospores were present.

A more convenient cultural method used for morphological studies was to place on a flamed slide in a Petri plate moist chamber a drop of radish decoction to which was added a small amount of mycelium from a young agar culture. Vegetative growth and sporulation occurred equally as well in such cultures as in the Petri plates. This method permitted microscopical examination without the necessity of removing the slide from the dish or disturbing the natural growth of the fungus.

In making the above described cultures, mycelium of different ages was used, care being taken to transfer as little as possible of the agar substratum with the mycelium. The age of the mycelium used to inoculate the drops was found to have a marked influence on zoospore production. When mycelium from a three- to four-day-old culture was used to inoculate the drop on the slide, zoospore production commenced within three to six hours. The young vegetative hyphae when removed from an abundant food supply and placed in the radish decoction apparently change very quickly into zoosporangia. On the other hand, when agar cultures 10 to 15 days old were used as a source of inoculum, zoosporulation usually occurred only after 24 to 48 hours. In the latter case, it was necessary for new vegetative growth to take place before zoosporangia were produced, since the old vegetative hyphae apparently do

not change into zoosporangia. In general it may be said that for typical and abundant zoospore formation, it is necessary to have vigorously growing vegetative hyphae in liquid culture in which there is little available food material.

MUCH BRANCHED ZOOSPORANGIA HAVE SPIRALLY CURLED DISCHARGE TUBES

The vegetative mycelium of the radish black-root fungus consists of rather long, moderately branched, hyaline, nonseptate hyphae. The hyphae often bear numerous short side branches which are profusely branched (Fig. 6 B) and which usually form a right angle with the main branch. The diameter of the vegetative hyphae varies from 8.2 to 11.3 microns (average 9.2).

Asexual reproduction is by means of zoospores produced in a single row in long profusely branched zoosporangia which are either terminal or intercalary in origin. Zoosporangia

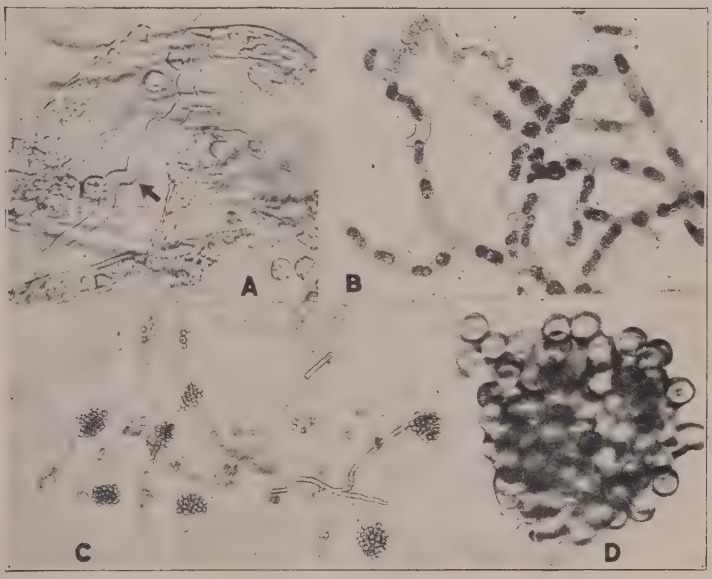


Fig. 7. Mode of production and liberation of the zoospores.

A. Empty, curled, tapering, discharge tube among vegetative hyphae in a water culture. Photomicrograph x 465.

B. Part of a large much branched zoosporangium containing the cylindrical granular zoospores. In the upper left corner is one of the spirally curled, tapering tips from which discharge of the zoospores takes place. Photomicrograph x 280.

C. Portion of large zoosporangium in water mount showing the spirally curled discharge tubes at the apices of which are clumps of recently liberated zoospores. Photomicrograph x 72.

D. Clump of recently discharged encysted zoospores at orifice of a discharge tube. Photomicrograph x 465.

are formed from the vegetative hyphae and are delimited, if terminal, by one crosswall and if intercalary, by two crosswalls. These crosswalls are located usually at the upper or lower axis of a side branch. The zoosporangia are differentiated from the vegetative hyphae by the fact that many of the lateral branches show a characteristic spiral curling (Fig. 7 A, B and C, Fig. 8 A, B and D). These are the hyphae from the apex of which the zoospores eventually escape, and will be designated hereafter as discharge tubes. The degree of curling varies from a wave-like form to a tight spiral twisting. The curling is often limited to a region a short distance back from the apex, but may extend the entire length of the discharge tube. In typical cases, there is a corkscrew-like region a short distance back from the apex of the discharge tube (Fig. 8 B). Often the discharge tube may double back and coil once or twice around itself near the spirally curled portion (Fig. 8 D). The discharge tubes show a gradual tapering towards the apex, the diameter at the tip being only about one-half that of the zoosporangial hyphae in general (Fig. 7 A and B).

ZOOSPORES FORMED BY SEGMENTATION OF THE GRANULAR CONTENT OF THE ZOOSPORANGIUM

As soon as the cross-walls delimiting the zoosporangium are laid down, the granular contents of the latter begin to segment into spores. The first evidence of spore formation is the appearance of narrow, transparent, transverse bands or planes across the protoplasm at regular intervals (Fig. 8 C). These become planes of separation, by means of which, in a few minutes, the entire contents of the zoosporangium are simultaneously segmented into distinct cylindrical protoplasmic bodies. In a few more minutes, one or more of the spirally twisted, tapering, discharge tubes are ruptured at the tip, and the zoospores, sliding along inside the hyphae, begin a steady migration to the nearest of these openings through which they escape into the surrounding medium. The cylindrical zoospores become very much elongated and constricted as they pass out the attenuated tip, but, once outside, quickly assume a globose shape and remain in a non-motile encysted state in a clump at the mouth of the discharge tube (Fig. 7 D and Fig. 8 D). The zoospores from the lateral hyphae which have no apical opening, pass backward into the main hyphae, often alternating with those of the main branch, as they join the procession toward the nearest discharge tube. It is not unusual for spores to become fastened in some of the tight spirals of the discharging tube and fail to pass out. Such spores usually round up in the spiral, but if a spore is caught in the constricted tapering tip, it retains its elongated form. If a weak killing solution is added to

a discharging zoosporangium, all movement is instantly arrested, and the zoospores usually round up within the zoosporangium (Fig. 8 E). The size and capacity of the zoosporangia vary. Some have been observed to discharge more than 300 zoospores while others have emptied less than 50.

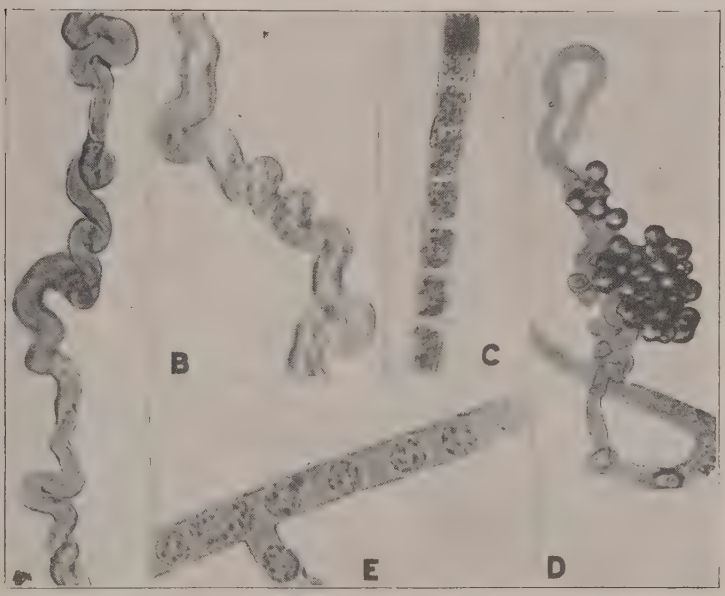


Fig. 8. Twisted condition of discharge tubes and details of zoospore formation.

A. Spiral twisting of lateral hypha through which zoospores are to be discharged. Photomicrograph x 385.

B. Spirally curled segment of zoosporangial hypha before zoospore formation. This will function as a discharge tube. Photomicrograph x 625.

C. Segment of a zoosporangial hypha showing the light colored transverse bands across the protoplasm, an early stage in zoospore formation. Photomicrograph x 665.

D. Clump of zoospores at the tip of a tapering, twisted, discharge tube. Photomicrograph x 525.

E. Segment of zoosporangium showing how the escaping zoospores round up inside the zoosporangium when their movement is stopped by adding a weak fixing solution. Photomicrograph x 625.

AFTER LIBERATION, ZOOSPORES CLING TOGETHER AT ORIFICE OF DISCHARGE TUBE. LATER SEPARATE, BECOME MOTILE

The clump of zoospores may remain at the orifice of the discharge tube for varying lengths of time or may break up immediately if the culture is disturbed. The encysted zoospores break away singly or in bunches, float around for awhile and come to rest. The diameter of the globose nonmotile encysted zoospores varies from 8.8 to 12.7 microns (average 10.2). Two to six hours after the zoospores are

discharged each escapes from its outer wall or cyst and swims away by means of two laterally attached cilia. After swimming about with a rapid whirling motion for varying lengths of time, the zoospore comes to rest, rounds up and germinates by sending out a slender germ tube.

SPHERICAL OOGONIA ACCOMPANIED BY ONE TO THREE ANTHERIDIAL TUBES

Sexual reproduction is by the usual method found in other Phycomycetes of this type. The spherical oogonia are terminal on short side branches, sometimes appearing almost sessile on the main hyphae (Fig. 9 A and C). They are globose and at first thin-walled, but soon show considerable thickening of the wall, the inner surface of which becomes wrinkled or scalloped as noted by Jones and Drechsler (11) in *Aphanomyces euteiches*. The irregular contour of the inner ooognial wall is plainly visible when the oospore is mature (Fig. 10 A). The contents of the oogonium are coarsely granular with a single large oil drop (Fig. 9 C) or occasionally two large oil drops (Fig. 9 B). The diameter of the oogonium varies from 32 to 44.9 microns (average 37.4).

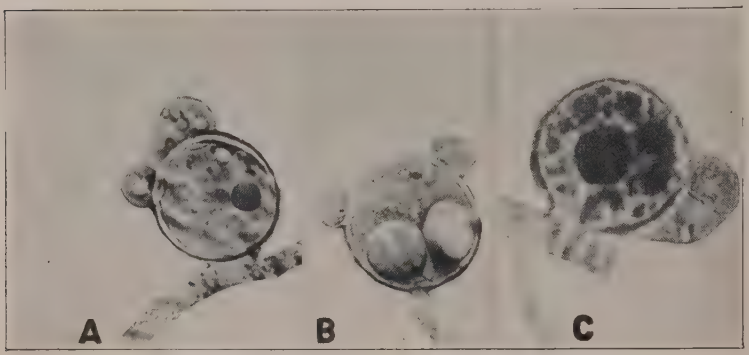


Fig. 9. Oogonia and antheridia of the black-root fungus.

A. Spherical oogonium with club-shaped antheridium closely appressed to its upper surface. The oogonium is almost sessile on the hypha and contains a large central oil drop. Photomicrograph x 600.

B. Stalked oogonium containing two large oil drops and showing on its upper surface the tips of two attached antheridia. Photomicrograph x 600.

C. Sessile oogonium showing one of the two attached antheridia. Photomicrograph x 600.

The antheridial stalk arises from the same hypha as the oogonial stalk, or from a hypha in close proximity to the oogonium, and often adheres closely to the oogonial stalk but has never been observed twining around it. The antheridial stalk may be unbranched, bearing a single club-shaped antheridium, but more frequently shows bifurcated branch-

ing at or near the base of the oogonium. Each of the two branches bears a club-shaped antheridium which lies closely appressed to the surface of the oogonium (Fig. 9 A and C). There are from one to three, usually two antheridia to each oogonium. In character and mode of origin the antheridia are very similar to those of *Aphanomyces euteiches* as described by Jones and Drechsler (11).

LARGE THICK-WALLED OOSPORE FORMED IN EACH OOGONIUM

Within the oogonium, there is formed a single globose oospore with a thick hyaline wall. The oospores vary in diameter from 21.4 to 29.8 microns (average 25.7). The thickness of the hyaline oospore wall varies from 2.5 to 4.5 microns (average 3.6). Although oospores in water and radish decoction cultures have been under observation until they were three months of age, no germination occurred.

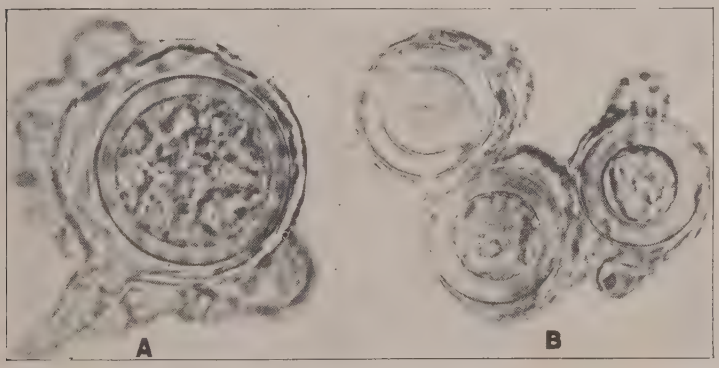


Fig. 10. Oogonia each containing a thick-walled oospore.

A. Oogonium containing a spherical thick-walled oospore. On the outer surface of the oogonium are two antheridia, one on each side. The inner surface of the oogonial wall is roughened or irregular in contour. Photomicrograph x 925.

B. A group of three oogonia, each containing a thick-walled mature oospore. Photomicrograph x 600.

BARRETT CORRECTLY ASSIGNED THE FUNGUS TO THE GENUS APHANOMYCES

The morphological characters of the fungus causing radish black-root show clearly that it is a species of the genus Aphanomyces, as Barrett (2) concluded in 1912. Most of the described species of Aphanomyces are saprophytes or are parasites on algae, but recent work by Jones and Drechsler (11) and by Linford (14) indicates that the genus includes other destructive root parasites.

PYTHIUM APHANIDERMATUM NOT ASSOCIATED WITH BLACK-ROOT

In 1915 Edson (7) described *Rheosporangium aphanidermatus* Edson as the cause of a damping-off of sugar beets and also as the cause of a black-root disease of radishes on the basis of inoculation experiments, details of which are not given. Upon the basis of Edson's work the present writer as well as others went on the assumption that the causal organism was *R. aphanidermatum*, or *Pythium aphanidermatum* (Edson) Fitz. as it was later classified by Fitzpatrick (8). However, the morphology of the causal organism isolated in these studies differed radically from that of *P. aphanidermatum* and showed that the fungus was a species of Aphanomyces.

Through the kindness of Dr. Charles Drechsler, a culture of *P. aphanidermatum* was secured for comparison with the organism associated with the radish disease under discussion. The extensive white aerial mycelial growth of *P. aphanidermatum* in artificial culture at once differentiated it from the flat mat-like growth of the radish Aphanomyces. The distinct morphological differences between these two genera made a morphological comparison unnecessary. In inoculation experiments, which will be described later, the culture of *P. aphanidermatum* failed to produce the black-root disease, while the disease was repeatedly produced by inoculation with the Aphanomyces species under discussion.

THE CAUSAL FUNGUS AN UNDESCRIBED SPECIES OF APHANOMYCES

The Aphanomyces species causing radish black-root does not seem to be identical with any of those previously described. The smooth wall of the oogonium differentiates it from the species with tuberculate or spiny oogonia, namely, *A. scaber* deBary, *A. stellatus* deBary, *A. coniger* Petersen, *A. parasiticus* Coker, *A. phycophilus* deBary and *A. norvegicus* Wille. The form of *A. laevis* described by Couch (5) in 1926 is differentiated from the Aphanomyces species in question herein by the brown color of the oogonia, the occasional occurrence of spiny oogonia, and the occasional, though rare, occurrence of an oogonium with two eggs. Of the described species, this leaves only three with smooth walled oogonia, *A. laevis* deBary, *A. helicoides* Minden and *A. euteiches* Drechsler, for further consideration.

According to von Minden (16), *A. helicoides* is closely related to *A. laevis*, and is differentiated primarily by the tendency of its antheridial stalks to wind around one another, around other nearby hyphae, or around the oogonium. There seems to have been some hesitancy on the part of von Minden in making a new species based on this difference, and Coker (4) is not sure that *A. helicoides* is different from *A. laevis*,

since he has found a strong tendency of the antheridial stalks of the latter to show this coiling habit. The fungus of the black-root disease has at no time shown any tendency towards a spiral or twining habit of the antheridial stalks.

There seems to be a lack of unanimity in the published descriptions of deBary's *Aphanomyces laevis*. The original description of *A. laevis* by deBary (3) contains very few morphological details upon which to base a comparison of the radish fungus. Humphrey (10) adds little to deBary's account. Peters (18) in his description of *A. laevis* found wide variations in the thickness of the oogonial and oospore walls which he attributed to environmental conditions and deemed not of sufficient importance to justify the creation of a new species. Coker (4, p. 161) noted a tendency of the antheridial stalks in *A. laevis* to twine around the oogonial stalks and for the antheridia to wrap themselves about the oogonia.

The fungus causing radish black-root differs from *A. laevis*, as described in the literature, in the greater diameter of its hyphae, zoospores, and oospores (with the exception of those given by deBary for the oospores), in the greater thickness and internal irregularity of the oogonial wall, in the lack of any twining tendency in the antheridial branches, and in the spiral curling of the zoosporangial discharge tubes.

In some respects, the organism causing radish black-root is very similar to *A. euteiches* Drechsler. Cultures of *A. euteiches* were kindly furnished by Dr. F. R. Jones and were compared with the radish organism. The latter differs from *A. euteiches* in its slower growth in culture, in the greater diameter of its hyphae and the preponderance of short side branches, and in the spiral curling of its zoosporangial discharge tubes. In addition to the above differences, inoculation tests, an account of which is presented later, have shown that *A. euteiches* does not produce the black-root disease.

The causal agent of radish black-root is apparently an undescribed species of Aphanomyces; and, because of its parasitic habit on *Raphanus sativus*, the binomial *A. raphani* n. sp. has been suggested (13). A technical description follows.

TECHNICAL DESCRIPTION OF APHANOMYCES RAPHANI N. SP., THE CAUSE OF RADISH BLACK-ROOT

The vegetative mycelium is hyaline, non-septate, and profusely branched at right angles, and bears short, conspicuous side branches. The hyphae are 8.2 to 11.3 microns (average 9.2) in diameter. The mycelium is slightly aerial in the early stages of growth on potato dextrose agar, but soon becomes a prostrate, dense, flat, tough mat.

The zoosporangia are terminal or intercalary, long and profusely branched, and differ from the vegetative hyphae

only in the spiral twisting of the branches from which the zoospores are discharged. The zoospores, which are produced in a single row in the zoosporangium by segmentation of its contents, are cylindrical, 6.9 to 26 microns in length and escape from one to several, tapering, more or less spirally twisted, lateral discharge hyphae. The zoospores become globose and encysted on emergence, are 8.8 to 12.7 microns (average 10.2) in diameter, and remain in a clump for a short time at the outlet of the discharge tube. The zoospores soon emerge from their cysts in motile form with two laterally attached cilia, swim about for a short period, come to rest, round up, and germinate by sending out a slender germ tube.

The oogonia are globose, 32 to 44.9 microns in diameter (average 37.4), and are terminal on short side branches and thick-walled when mature. The oogonial wall has a smooth outer surface and an irregularly contoured inner surface. The antheridia are produced on simple or bifurcated slender stalks which arise from the hypha bearing the oogonial stalk or from a near-by hypha. The antheridial stalk is often closely associated with the oogonial stalk but never twines about it. The antheridia are club-shaped, typically one to three, usually two to each oogonium, and lie flat on the surface of the oogonium. The oospores are single, globose, 21.4 to 29.8 microns (average 25.7) in diameter, with a hyaline wall 2.5 to 4.5 microns thick. Germination of the oospores has not been observed.

The fungus is parasitic on *Raphanus sativus* L. Type locality: Lafayette, Ind.

BLACK-ROOT FUNGUS GROWS BEST AT *23°-27°* C. (*73°-81°* F.)

The causal organism of radish black-root grows throughout a rather wide range of temperatures. This is indicated by the fact that the disease occurs in Indiana from early spring throughout the summer. It has also been observed that the disease, under Indiana conditions, tends to become more severe as the season advances. This indicates that a fairly warm soil temperature is favorable for the development of the disease. Laboratory studies have shown that a rather high temperature is most favorable for the vegetative development and sporulation of the fungus.

Three series of cultures were grown at different temperatures to determine the optimum temperature for the mycelial development of the fungus. A small square from an agar plate culture was planted in the center of each of a number of poured plates of potato dextrose agar. These plates were then incubated at 25°C. for 24 hours to allow the fungus to start development from the transplanted blocks of agar. After this preliminary incubation period, the plates were placed in

moist chambers and incubated at different temperatures. At the end of 8 days the mean increase in colony diameter at 9°C. was 0 mm., at 12-13°, 20 mm., at 15°, 39 mm., at 20°, 53 mm., at 23°, 73 mm., at 27°, 71 mm., at 30°, 31 mm., and at 35°, 0 mm.

The results show that mycelial development of the fungus takes place at a wide range of temperatures, but the maximum development occurs at 23° to 27°C. The fact that no growth occurred at 9° and 35°C. indicates that low and excessively high temperatures are unfavorable for the growth of the fungus.

A series of liquid cultures, made on slides as previously described, were placed in a series of incubators at the following temperatures: 11°-12°, 20°, 25°, 27.5° and 35°C. After two days incubation, a few zoospores were present in the cultures held at 11°-12° and 25°C. In three days, there was an abundance of zoospores at 20° and 25°C. but no increase in the number present at 11°-12°C. There were a few zoospores at 27:5°C. but none at 35°C. A temperature of 20° to 25°C. was most favorable for zoospore production. Oogonia and antheridia were present at 20° and 25°C. after three days incubation.

FUNGUS ENTERS HOST THROUGH NATURAL WOUND MADE BY EMERGENCE OF SECONDARY ROOT

In a previous publication (12) it has been pointed out that in practically every case out of many studied, the fungus causing radish black-root apparently had entered the host at the natural wound made where the secondary root emerged from the tissues of the primary root. This theory has been substantiated by many observations since that time. The secondary roots, which are in two opposite rows along the primary root, originate in the inner part of the pericycle and push out through the outer tissues of the primary root (Fig. 11 A). In so doing, they produce a natural wound in the primary root which may serve as an avenue for the entrance of the fungus. That the fungus enters at this wound and not through the secondary root itself is indicated by the fact that, in the early stages of the disease, the secondary root at the center of an incipient lesion presents a bright healthy appearance (Fig. 4 C and Fig. 11 B). Free-hand sections as well as microscopic examination have given evidence of the progress of the fungus from this natural wound out into the adjoining tissues of the main root, but never into the secondary root, at least in the early stages of the disease.

In order to determine more accurately the exact point of origin of the black-root lesions, a large number of young White Icicle radishes grown in infested soil were examined under a binocular microscope. A total of 81 diseased roots

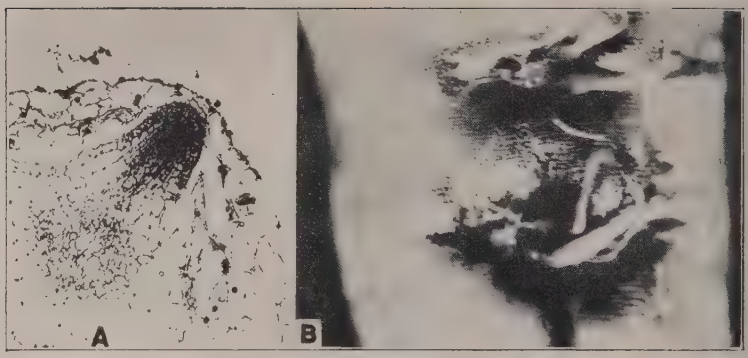


Fig. 11. The natural wound produced by the emergence of a secondary root permits the entrance of the black-root fungus.

A. Transverse section of a seedling radish root, stained by the triple stain, showing the mode of emergence of a secondary root. The tissues of the primary root are ruptured by the emerging root and it is through this natural wound that infection usually takes place. Photomicrograph x 45.

B. Segment of primary root enlarged x 10 to show two incipient blackened lesions around the bases of white, healthy secondary roots.

were examined, and of the 368 incipient lesions present on these roots, 349, or 94.8 per cent, were located at the bases of secondary roots. The remaining 19 incipient lesions, or 5.16 per cent, apparently had their origin at growth cracks or some other type of injury. In subsequent studies, hundreds of young roots have been examined and 95 to 100 per cent of the incipient lesions have been found to be at the bases of the secondary roots.

The hypocotyl, petiole and leaf infection in seedling radishes apparently has its origin in the hypocotyl tissues at the surface of the soil. Such infection has always been first noted at or near the juncture of the hypocotyl and cotyledon petioles whence, presumably, it may progress down into the hypocotyl and up into the petiole tissues. No actual connection has been traced between this cotyledon and upper hypocotyl infection and the characteristic infection in the edible portion. The avenue for the entrance of the fungus into the upper hypocotyl and petiole tissues has not been determined.

FUNGUS PENETRATES THE SPACES BETWEEN THE CELLS

In free-hand razor sections, the course of the fungus can readily be traced from the ruptured host tissues at the base of the secondary root into the inner tissues of the main root (Fig. 12B). An incipient lesion appears as a small dark brown area in the ruptured tissues at the side of the secondary root (Fig. 12 A).

Stained sections from paraffin material as well as unstained free-hand sections have been used to study the relation of

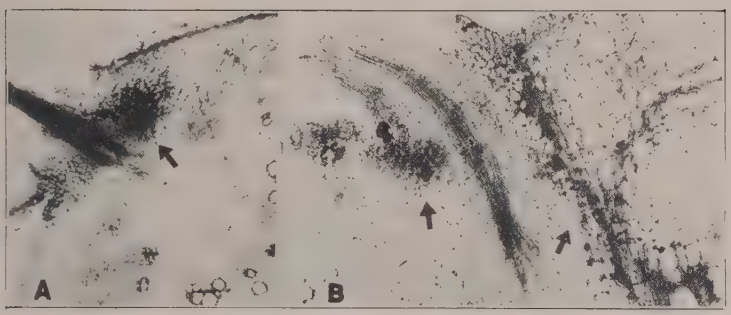


Fig. 12. Transverse sections of young radish roots showing the progress of the black-root fungus into the tissues.

A. Unstained free-hand cross-section of young radish root through the base of a secondary root. The presence of the fungus is indicated by the blackened tissues to which the arrow points at one side of the base of the secondary root, where the latter, when it emerged, ruptured the tissues of the primary root. Photomicrograph x 15.

B. Unstained free-hand cross-section through a lesion at the base of secondary root showing the path followed by the fungus as indicated by the dark discoloration extending down along the side of, but not within, the secondary root. Arrows point to the diseased tissues. Photomicrograph x 50.

the fungus to the host tissue. The mycelium is not abundant. In free-hand sections the intercellular spaces containing the fungus seem to be packed with a dark brown granular mass and the adjacent cell walls are likewise dark brown (Fig. 12 B). The large colorless hyphae are in the intercellular spaces and apparently cause no separation or disintegration of

Fig. 13. Sections of diseased root tissue greatly magnified to show the location of the fungus between the cells of the root tissue. (Sectioned and stained by D. C. Cooper.)

A. Portion of transverse section of diseased radish root showing dark granular hyphae between the cells. Triple stain. Photomicrograph x 290.

B. Portion of longitudinal section of a diseased radish root showing the large hyphae in an intercellular space. The adjacent cells show, as yet, little or no injury. Triple stain. Photomicrograph x 535.

the walls of the host cells (Fig. 13 A and B). That the fungus does not seriously injure the host cell wall is indicated by the more or less normal turgid appearance of the cells adjacent to invaded intercellular spaces.

IN THE LESION, NOT ALL INTERCELLULAR SPACES INVADED

The distribution of the fungus in the tissues is indicated by dark brown granular intercellular spaces scattered irregularly throughout the intervening normal tissue. In the sections, the fungus is seen to occupy single intercellular spaces or groups of intercellular spaces entirely surrounded by healthy tissue. Except in old very black lesions, the mycelium is rather scanty in the tissues and a relatively small proportion of the intercellular spaces is occupied. Invasion of all the intercellular spaces occurs only in the severely infected tissues of old lesions and the extent to which the infected tissue is blackened may be taken as a measure of the extent to which the intercellular spaces are occupied.

OOSPORES OCCASIONALLY FOUND IN OUTER TISSUES

In a few instances, oospores have been found sparingly in the outer layer of disintegrated tissues of badly diseased roots. Oospores have not been found elsewhere in the host tissues, nor have zoosporangia been found in the host tissues.

DISEASE READILY PRODUCED BY GROWING RADISHES IN STERILIZED SOIL INOCULATED WITH THE FUNGUS

Numerous greenhouse and field tests have been made to determine the pathogenicity of the fungus and the relative susceptibility of different radish varieties. Many successful inoculations have been made with pure cultures of the organism in sterile soil and numerous reisolations have been made. For inoculation studies, four-inch or six-inch pots were filled with greenhouse soil and autoclaved for four hours at 25 to 30 pounds pressure. Inoculum was secured by growing the fungus five to eight days in a radish leaf decoction in a Petri plate or in a poured plate of potato dextrose agar. The mycelial growth was cut into shreds and along with the culture medium was added to flasks of sterile water. In making inoculations, the upper half-inch of soil was removed and enough of the inoculum added to wet the surface of the soil. Radish seeds were then planted on the wet surface and the soil that had been removed was scattered over the seed. The pots were placed on greenhouse benches and the soil was kept at a relatively high moisture content. As soon as the majority of the plants had reached the stage when the roots begin to enlarge, they were pulled, washed, and examined for root infection. Reisolations were made from the lesions in each series of inoculations, the identity of the organism was determined, and in many cases its pathogenicity was again proved.

SEEDLING LEAVES INFECTED IN INOCULATION TESTS

On March 18, 1926, a series of inoculations were made by the method outlined above and two varieties of radishes were used, White Icicle and White Chinese. A similar series of pots with no inoculum served as a control. Before root enlargement had begun the plants were examined for hypocotyl, petiole and leaf infection without removing them from the soil. Many of the inoculated plants showed evidence of such infection in the shape of dark streaks running up from the juncture of the hypocotyl and petioles through the cortical tissues of the long petioles of the cotyledons and true leaves. Such streaks often extended into the mid-rib as well as the smaller veins of the leaf and caused a lateral curling of the latter. The causal fungus was reisolated from these petiole and leaf infections. The plants were allowed to grow until April 22 when they were pulled and examined for root infection. The results of the two examinations are presented in Table 1.

TABLE 1.—Results of greenhouse tests with soil inoculated with *Aphanomyces raphani*

VARIETY	INOCULATED POTS				CONTROL POTS	
	Hypocotyl, petiole, and leaf infection		Root infection			
	Number plants	Percentage infected	Number plants	Percentage infected	Number plants	Percentage infected
White Icicle	217	36.5	199	32.4	107	0
White Chinese	97	24.7	58	26.0	63	0

The total number of plants at the second examination was smaller because some of the plants that had previously shown severe hypocotyl, petiole and leaf infection had died in the meantime. Even though there seems to be a close correlation between the percentage of infection in the aerial parts of the plant and in the roots, the two types of infection were not confined to the same plants. Some plants showed severe root infection with no evidence of petiole and leaf infection and vice versa.

The results presented in this table show that the percentage of infection in the White Chinese variety was somewhat lower than in the White Icicle. However, the infection percentage alone is not in this case an adequate measure of the relative susceptibility of the two varieties because on the White Icicle the lesions were numerous and severe while on the White Chinese the lesions were few and inconspicuous.

OTHER SUSPECTED FUNGI FAIL TO PRODUCE THE DISEASE

Inoculations with *Pythium aphanidermatum* (secured from Dr. Chas. Drechsler) upon the varieties White Icicle and Early

Scarlet Globe were made simultaneously with those reported in Table 2. In these tests there was a total of 22 White Icicle and 58 Early Scarlet Globe radishes which grew normally and produced large edible roots and none showed evidence of the disease when examined.

The White Icicle variety of radish was used in an additional set of pot inoculation tests on June 1, 1926, in which the pots were divided into three parallel series: one inoculated with the radish fungus, one inoculated with *Pythium aphanidermatum*, and one with no inoculum to serve as a control. Of the 106 radishes which grew in the pots inoculated with the radish fungus, 100 per cent showed severe black-root infection, while none of the 53 radishes which grew in the soil inoculated with *P. aphanidermatum* showed any lesions. The controls were all healthy.

Along with these inoculations, tests were made with two strains of *Aphanomyces euteiches* secured from Dr. F. R. Jones. The very susceptible White Icicle variety of radishes was used and the plants grew normally and produced large vigorous roots. Of the 228 plants that were pulled, washed and carefully examined for root infection, none showed any black-root lesions.

SUSCEPTIBILITY OF DIFFERENT VARIETIES IN GREENHOUSE TESTS

In order to test the susceptibility of different varieties of radishes to the disease, 18 seedsmen's varieties were planted on April 15, 1926, in pots of sterilized inoculated soil. The plants were pulled, washed and examined on May 26. Only root infection was recorded in this test, although infection of the above ground parts occurred to some extent. The results are presented in Table 2.

TABLE 2.—Susceptibility of radish varieties to *Aphanomyces raphani* under greenhouse conditions

VARIETY	TYPE	INOCULATED POTS		CONTROL POTS (not inoculated)	
		Number Plants	Percentage infected	Number Plants	Percentage infected
White Icicle	Long white	50	96.0	44	0
Crimson Giant	Red globe	27	85.1	12	0
Early Scarlet Turnip	Red globe	30	80.0	12	0
Saxa	Red globe	40	77.5	37	0
White Delicious	Long white	44	77.3	39	0
Giant White Stuttgart	White globe	30	76.6	29	0
White Strasburg	Long white	41	75.6	24	0
Early Snowball	White globe	44	75.0	45	0
Model White Box	White globe	41	70.9	37	0
French Breakfast	Red oval	22	68.2	27	0
White Lady Finger	Long white	50	68.0	45	0
Livingston's Pearl	Long white	59	64.5	51	0
Early Scarlet Globe	Red globe	47	63.8	48	0
Large White Summer Turnip	White globe	38	58.0	35	0
Cincinnati Market	Long red	42	57.1	42	0
Cooper's Sparkler	Red globe	21	57.1	31	0
White Chinese	Long white	16	37.5	10	0
Chinese Rose	Long red	25	16.0	11	0

All but two of the 18 varieties of radishes proved very susceptible to the disease. The two winter varieties, White Chinese and Chinese Rose, not only showed a lower percentage of infection than the summer varieties, but also were much less severely diseased. As was usually the case, the white varieties showed the disease in a form more severe than did the others.

SUSCEPTIBILITY OF DIFFERENT VARIETIES IN BADLY INFESTED GARDEN SOIL

On April 19, 1923, 22 varieties of radishes were planted in a home garden in Lafayette that was known to be badly infested with the black-root fungus. No damping-off effects were noted, but there was considerable hypocotyl, petiole and leaf infection. On June 8, the radishes were pulled, washed and examined for evidence of the disease on the roots. The results are presented in Table 3.

TABLE 3.—Susceptibility of radish varieties to *Aphanomyces raphani* in severely infested garden soil, 1923

VARIETY	Type	Number plants	Percentage infected
Brightest Long Scarlet	Long red	34	100
Early Scarlet Turnip	Red globe	35	100
Half-long Deep Scarlet	Long red	32	100
White Lady Finger	Long white	57	100
Livingston's Pearl	Long white	37	94.6
Early Golden Yellow Oval	Yellow globe	40	90
Early Long Scarlet Short Top	Long red	28	89.3
Cincinnati Market	Long red	85	88.2
Crimson Giant	Red globe	37	86.5
Cooper's Sparkler	Red globe	32	84.3
White Strasburg	Long white	45	80.0
Improved Chartier	Long red	19	78.9
Golden Globe	Yellow globe	42	76.4
White Icicle	Long white	53	75.5
Early Scarlet Globe	Red globe	40	75.0
Model White Box	White globe	51	70.6
Early Snowball	White globe	75	57.3
Giant White Stuttgart	White globe	46	56.5
Large White Summer Turnip	White globe	38	55.3
Long Black Spanish	Long black	not counted[1]	50+
Round Black Spanish	Black globe	not counted[1]	50+
White Chinese	Large white	60	0

[1] Counts were not made because of the difficulty in recognizing incipient lesions on the black roots.

In this test, only the White Chinese variety showed immunity to the disease. The amount of infection varied from 50 to 100 per cent in the other 21 varieties. The percentage of infection in the different varieties does not, however, adequately express the relative severity of the disease. Some varieties of the red globe type showed a higher percentage of infection than some of the long varieties, but in the former the lesions were usually confined to the long slender root and the enlarged edible portion was not involved, while, in the long type, lesions usually occurred on all parts of the enlarged edible portion of the root. This difference in the severity

of the disease on the different types was in part due to the location of the secondary roots, as has been pointed out elsewhere, and in part to a general tendency of the red varieties to be less severely diseased.

CLEAN SOIL INOCULATED WITH SOIL FROM INFESTED GARDEN

In early May, 1923, two bushels of soil from a garden in which radish black-root had occurred in a severe form during the preceding three years was scattered thinly over a plot of ground about 18 by 40 feet in size in an experimental garden. A similar plot to which no inoculum was added was used as a control. On May 22, 23 varieties of radishes were planted in each plot. The radishes were pulled and examined for infection on June 19 and the results are given in Table 4.

TABLE 4.—Susceptibility of radish varieties to *Aphanomyces raphani* in recently inoculated garden soil, 1923

VARIETY	Type	INOCULATED SOIL		UNINOCULATED SOIL	
		Number plants	Percentage infected	Number Plants	Percentage infected
Livingston's Pearl	Long white	50	26.0	42	0
Early Long Scarlet Short Top	Long red	44	16.0	71	0
Golden Globe	Yellow globe	75	13.3	49	0
White Icicle	Long white	8	12.5	74	0
White Lady Finger	Long white	70	11.4	76	0
Early Golden Yellow Oval	Yellow globe	109	11.0	149	0
Early Scarlet Globe	Red globe	144	9.7	43	0
Round Black Spanish	Black globe	67	7.4	39	0
Long Black Spanish	Long black	58	6.9	75	0
Crimson Giant	Red globe	112	5.3	15	0
Model White Box	White globe	98	5.1	96	0
Large White Summer Turnip	White globe	71	4.2	68	0
Improved Chartier	Long red	50	4.0	90	0
Cincinnati Market	Long red	131	3.0	95	0
Brighest Long Scarlet	Long red	41	2.4	38	0
Early Snowball	White globe	137	2.2	114	0
Cooper's Sparkler	Red globe	31	0	42	0
Early Scarlet Turnip	Red globe	87	0	89	0
Giant White Stuttgart	White globe	63	0	81	0
Half-Long Deep Scarlet	Long red	158	0	143	0
Sakurajima	Yellow oval	46	0	20	0
White Chinese	Long white	101	0	66	0
White Strasburg	Long white	110	0	60	0

The results presented in Table 4 show that 16 of the 23 varieties contracted the disease, and, since no infection occurred in the adjacent control plot, that the causal fungus was introduced with the infested soil, and consequently may be carried from place to place by this means.

That the low percentages of infection in this plot were not due to climatic influence is proved by the fact that very high percentages of infection occurred in a home garden in the same locality, as shown in Table 3. Therefore, it seems safe to conclude that this low incidence of infection is due to a paucity of inoculum, inasmuch as the plot had just been inoculated and the fungus had not had time to increase in the soil.

The plot used in 1923 was again used for radishes in 1924 without further inoculation. Eleven varieties were planted on April 25 and pulled for examination June 5 to 11. The results are presented in Table 5.

Table 5 shows that there was a higher incidence of the disease in 1924 than in the previous year, presumably because there had been a great increase of infective material in the soil subsequent to its inoculation the year before. The percentage of infection was much higher in some of the globe types than in the long types, but, as in previous tests, the disease was more severe on the long types. The White Chinese variety remained free from infection.

A large number of radish varieties were again grown in this plot in 1925, but due to the writer's absence on leave no records were obtained. On May 19, 1926, however, 14 varieties of radishes were planted in the same plot along with eight rows of garden peas. The peas were grown to maturity and were examined at frequent intervals, but no evidence of a diseased condition of the roots was found. The radishes were pulled and examined for black-root on June 16 and the results are presented in Table 6.

TABLE 5.—Susceptibility of radish varieties to *Aphanomyces raphani* in soil one year after inoculation, 1924

VARIETY	Type	Number plants	Percentage infected
Round White	White globe	175	74.2
Long Red	Long red	58	67.2
Improved Chartier	Long red	111	63.0
White Strasburg	Long white	73	34.2
White Icicle	Long white	266	29.0
White Lady Finger	Long white	121	28.1
White Delicious	Long white	117	16.2
Cincinnati Market	Long red	139	15.8
Saxa	Red globe	126	10.3
Scarlet Turnip White Tip	Red globe	130	10.0
White Chinese	Long white	23	0

TABLE 6.—Susceptibility of radish varieties to *Aphanomyces raphani* in inoculated soil in which radishes had been grown three years in succession, 1926

VARIETY	Type	Number plants	Percentage infected
Livingston's Pearl	Long white	73	100
White Icicle	Long white	113	100
White Lady Finger	Long white	71	100
White Delicious	Long white	77	93.5
White Strasburg	Long white	74	93.2
Giant White Stuttgart	White globe	54	66.6
Large White Summer Turnip	White globe	79	64.5
Early Scarlet Globe	Red globe	94	53.1
French Breakfast	Red oval	58	50.0
Early Snowball	White globe	70	41.4
Model White Box	White globe	110	31.8
Cincinnati Market	Long red	83	25.3
Saxa	Red globe	90	20.0
White Chinese	Long white	62	9.6

The results in Table 6 show that the disease was much worse than in either of the two previous years when records were taken. Not only was the percentage of infection much higher, but the effects of the disease were more severe, especially on the white types. In the White Chinese variety, which had shown immunity in all previous field tests, 9.6 per cent of the plants were infected, but this infection was confined to a few very small lesions at the bases of the secondary roots.

WHITE CHINESE VARIETY SHOWS RESISTANCE

The foregoing field and greenhouse tests have demonstrated the pathogenicity of the radish black-root fungus to 30 seedsmen's varieties of radishes, including all types. Only the White Chinese, a winter variety, has shown any consistent evidence of resistance to the disease.

DISEASE MOST DESTRUCTIVE ON LONG WHITE VARIETIES

The disease is less severe on red than on white radishes. Apparently the fungus invades the tissues of the white varieties much more rapidly and extensively than it does the tissues of the red varieties. The somewhat greater susceptibility of the white radishes in general and the presence of avenues of infection on the edible portion of the long type result in the disease being especially destructive to long white radishes.

OTHER ROOT CROPS NOT SUSCEPTIBLE

In addition to the radish varieties, the following root crops have been grown in field plots where the radish black-root fungus was present: Six varieties of turnips, five varieties of carrots, two varieties of parsnips, two varieties of peas, garden beets and yellow mangels. No evidence has been found that any of these root crops are subject to the disease, and no host other than the radish has been found for the fungus.

CONTINUOUS CROPPING INCREASES THE SEVERITY OF THE DISEASE

The results in Tables 4, 5 and 6 show that the incidence of the disease was greater the second year than the first year after soil inoculation and much greater the fourth year than the second. The most logical explanation of this increase in the amount and severity of the disease seems to be that there was a gradual increase of the inoculum in the soil from year to year.

That soil moisture was not the factor responsible for the increase in the severity of the disease seems a logical conclusion in view of the fact that each season the radishes were irrigated by an overhead irrigation system and a rather high soil moisture content was maintained at all times.

That temperature was not the determining factor also seems likely, since the records of the local station of the U. S. Weather Bureau show that the mean temperature for the period between the time of planting the seeds and harvesting the radishes in 1923 was 70.1°F.; in 1924, 57.4°F.; and in 1926, 66°F. Thus, the only season in which the optimum temperature for the fungus (73°-81°F.) was approximated was the first season, the season in which the incidence of the disease was lowest, while the season in which the disease appeared in its greatest severity (1926), the mean temperature lacked 7° of approaching the lower limits of the optimum temperature. Furthermore, that the first year, 1923, was favorable to the disease was proved by the heavy infection occurring in garden soil as shown in Table 3. Therefore, it seems likely that the increased severity of the disease from year to year was due to an increase in the inoculum present in the soil and not to environmental factors.

DISEASE PERSISTS MANY YEARS IN THE SOIL

That the parasite which causes radish black-root is a soil fungus which lives over winter and persists in the soil has been repeatedly demonstrated by its presence year after year in many home gardens and in the experimental plots at Lafayette, and by the fact that successful inoculation of uninfested soil has been accomplished by using as inoculum soil from a badly-infested garden. Oospores are produced in the old rotted tissues and may possibly be instrumental in carrying the fungus over winter. The disease has been known to occur in Lafayette in gardens on what might be termed virgin soil. The gardens were made in a place which had been in sod for many years, and black-root appeared on radishes the first year the soil was used for a garden. Not all soils have been found infested, but apparently the causal agent is a widely-distributed soil organism.

NOT A SEED CARRIED DISEASE

Little is known concerning the dissemination of the disease other than that it is carried in the soil from infested areas. No evidence that the disease is seed-borne has been obtained by planting large numbers of seed from widely-separated sources in sterile soil. Under these conditions the disease has never occurred. The possibility of the existence of wild or weed hosts of the fungus has not been investigated.

RED GLOBE RADISHES ESCAPE SERIOUS INJURY

No adequate control measures for this disease are known. None of the popular varieties are resistant to the disease. However, the nature of the infection on the different types of

radishes offers some relief where the fungus is prevalent in the soil. As has been previously pointed out, the infection on the globe type of radishes usually occurs on the long slender root and in the red globe radishes seldom reaches the enlarged edible portion. On the other hand, infection of the long type of radishes takes place directly into the enlarged edible portion of the root. Owing to the rapid penetration of the fungus into the white radishes, they are rendered useless for table purposes. On badly infested soil it is often possible to produce a satisfactory crop of the red globe type of radishes, while the long type, especially the long white, is almost sure to be a total failure.

Early planting to avoid hot weather may often prove of value in combating this disease. Sterilization of the soil has not been tried, but steam sterilization would very likely control the disease where such a method could be utilized.

LITERATURE CITED.

1. Artschwager, Ernst. Anatomy of the vegetative organs of the sugar beet. Jour. Agr. Res. 33:143-176. 1926.
2. Barrett, J. T. A serious root disease of radish. Abs. in Phytopath. 2:96. 1912.
3. Bary, A. de. Einige neue Saprolegnieen. Jahrb. Wiss. Bot. (Pringsheim) 2:169-192. 1860.
4. Coker, W. C. The Saprolegniaceae with notes on other water molds. 201 pages, Chapel Hill, N. C. 1923.
5. Couch, J. N. Notes on the genus Aphanomyces, with a description of a new semiparasitic species. Jour. Elisha Mitchell Sci. Soc. 41:213-225. 1926.
6. Edson, H. A. Seedling diseases of sugar beets and their relation to root-rot and crown-rot. Jour. Agr. Res. 4:135-168. 1915.
7. Edson, H. A. *Rheosporangium aphanidermatus*, a new genus and species of fungus parasitic on sugar beets and radishes. Jour. Agr. Res. 4:279-291. 1915.
8. Fitzpatrick, H. M. Generic concepts in the Pythiaceae and Blastocladiaceae. Mycologia 15:166-173. 1923.
9. Gardner, Max W. Indiana plant diseases, 1921. Proc. Ind. Acad. Sci. 33 (1923):163-201. 1924.
10. Humphrey, J. E. The Saprolegniaceae of the United States with notes on other species. Trans. Amer. Phil. Soc. (N. R.) 17:63-148. 1893.
11. Jones, F. R. and Drechsler, Charles. Root rot of peas in the United States caused by *Aphanomyces euteiches* (n. sp.) Jour. Agr. Res. 30:293-325. 1925.
12. Kendrick, James B. Infection-court in radish black-root. Abs. in Phytopath. 14:66. 1924.
13. Kendrick, James B. Radish black-root—caused by *Aphanomyces raphani* n. sp. Abs. in Phytopath. 17:43. 1927.
14. Linford, Maurice B. Additional hosts of *Aphanomyces euteiches*, the pea root-rot fungus. Phytopath. 17:133-134. 1927.
15. Link, G. K. K. and Gardner, Max W. Market pathology and market diseases of vegetables. Phytopath. 9:497-520. 1919.
16. Minden, M. von. Pilze 1. Phycomycetes. Aphanomyces. Kryptogamenflora der Mark Brandenburg 5:555-562. 1912.
17. Percival, John. Agricultural botany. 828 pages, London. 1910.
18. Peters, L. Uber die Erreger des Wurzelbrandes. Arb. K. Biol. Anst. Land-u. Forstw. 8:211-259. 1911.